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ORIGINAL ARTICLE

Epstein–Barr virus seroprevalence and viral load at disease onset in children with inflammatory bowel disease

Gaël Kornitzer,[*,†] Michelle Rosenstein,[*,‡] Marie-Catherine Turcotte,[*,†] David Godin,[*,§] Véronique Groleau,[*,†,§] Christian Renaud,[*,§,¶] Fabien Touzot,[*,§,‖] Prevost Jantchou,[*,†,§] Philippe Ovetchkine[*,§,¶] and Colette Deslandres[*,†,§]

*Faculty of Medicine, University of Montreal, [†]Division of Gastroenterology, Hepatology and Nutrition, Department of Paediatrics, [‡]Division of Paediatrics, Department of Paediatrics, [§]Research Center, [¶]Division of Infectious Diseases, Department of Paediatrics and [‖]Division of Immunology, Department of Paediatrics, CHU Sainte-Justine, Montreal, Canada

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Correspondence
Colette Deslandres, CHU Sainte-Justine, 3175 chemin de la Côte Sainte-Catherine, Montréal, QC, Canada.
Email: colette.deslandres@umontreal.ca

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Abstract

Background and Aim: Patients with inflammatory bowel disease (IBD) are at increased risk for life-threatening complications of Epstein–Barr virus (EBV), including lymphoproliferative diseases. These complications are likely related to inherent immune dysfunction and immunomodulating therapies often used. We aimed to determine the seroprevalence of EBV at diagnosis in our population, its impact on disease at onset, and the risk of active EBV infection.
Methods: We included patients newly diagnosed with IBD for whom an EBV serology was performed over a 2-year period. Demographic information and data on disease characteristics were collected retrospectively. Stored serum from the time of diagnosis was retrieved when available for the patients with positive EBV serology, and quantitative polymerase chain reaction testing was performed to assess the pretreatment viral load of EBV.
Results: One hundred twenty patients were included in the study. Fifty-three patients (44.2%) had positive EBV serology at diagnosis. Stratified by age group, the prevalence of seropositive patients was for 0 to <10 years 35%, 10 to <17 years 46%, and ≥17 years 50%. Overall, therapies started within 6 months of diagnosis were similar in both the seropositive and seronegative groups. Within the seropositive group, 66% received systemic corticosteroids, 32.1% infliximab, 5.7% adalimumab, and 5.7% azathioprine.
Conclusion: EBV seroprevalence is high in pediatric patients with IBD. EBV seropositivity did not seem to influence the severity of disease at onset or initial choice of therapy.

Introduction

Patients with inflammatory bowel disease (IBD), like other immunosuppressed patients, are at a higher risk for complications related to Epstein–Barr virus (EBV) infection than the general population. Infection with EBV in patients with IBD has been associated, much like cytomegalovirus and other viral infections, with disease flare-ups or exacerbation of symptoms. There has also been some literature suggesting a role for EBV in disease onset and severity at presentation, as well as other forms of colitis or enteritis mimicking IBD.[1–4] Most concerning, however, is the reported increased risk of various neoplastic and lymphoproliferative disorders (LDs), including post-transplant LD (PTLD), hepatosplenic T-cell lymphomas (HSTCL), intestinal lymphomas, and hemophagocytic lymphohystiocytosis (HLH), as well as a case of lymphomatoid granulomatosis in our center reported by Destombe *et al*. in 2010.[5–10]

It has been shown that specific therapies for IBD may exacerbate the risk of lymphomas and other LDs in these patients. Lymphoproliferative and malignant complications of primary EBV infection have been associated specifically with the use of immunomodulators, namely thiopurines, both as monotherapy and in combination with antitumor necrosis factor (TNF) agents.[5–9,11] Despite initial reports of increased risk of malignancy with anti-TNF use, more recent, larger studies, including the DEVELOP cohort, which prospectively followed 5766 pediatric patients with IBD, have shown no association between increased risk of malignancy of LDs with anti-TNF in monotherapy.[12] Many studies, both retrospective and prospective, continue to support the association between thiopurine exposure and malignancy in these patients.[6,12–14] Due to these concerns and the specific implication of primary EBV infection, previous studies have focused primarily on the risk of seroconversion in EBV naïve patients with IBD treated by immunomodulators. These studies have mainly focused on adult populations. There are very few studies in the literature on the seroprevalence of EBV in patients with IBD, and those that exist are often cross-sectional studies. Hradsky *et al*. reported a seroprevalence of 64% in pediatric patients with IBD, with detectable viral loads in 14% of these patients at any time during their follow-up. They report a positive association between higher viral load and anti-TNF therapy as well as increased risk for early seropositivity in patients treated with azathioprine.[15] More recently, Bachman *et al*. and Harris *et al*. demonstrated seroprevalence of 53% at the time of diagnosis in pediatric IBD patients and seroconversion rate of 17% in initially EBV-negative patients.[16,17]

It has increasingly become common practice for pediatric gastroenterologists to assess seropositivity for EBV at the time of diagnosis of IBD. The goal of this clinical practice is to prevent possible complications of thiopurine therapy, such as lymphomas and other LPDs, in seronegative patients who are at risk of developing a primary EBV infection during their treatment. Thus, this EBV seropositivity can often inform the initial choice of therapy and may lead to favoring other therapies over use of azathioprine. For these reasons, clinical practice guidelines from both the Canadian Association of Gastroenterology (CAG) and the European Crohn's and Colitis Organization (ECCO) suggest that clinicians consider assessing EBV serology prior to immunomodulatory therapy.[18,19] The primary aim of this study is to describe the seroprevalence of EBV in our local pediatric patient population at the time of diagnosis and to evaluate the relationship of EBV seropositivity with disease characteristics at the time of diagnosis. Our secondary aim was to retrospectively assess the pre-treatment viral load at the time of diagnosis.

Methods

All patients aged 0–18 years with a new diagnosis of Crohn's disease, ulcerative colitis or IBD-unclassified over a 2-year period from January 2016 to December 2017, inclusively, were identified from a local database and included in the study. This database is comprehensive and is maintained to include all patients being followed for IBD in a tertiary care pediatric hospital (Sainte-Justine University Hospital Center).[20] The study was approved by the Research Ethics Board of the CHU Sainte-Justine in 01/2019. Diagnosis of all patients was made on the basis of endoscopic and histologic findings. Anti-viral capsid antigen (VCA) IgG or anti-Epstein–Barr nuclear antigen (EBNA) IgG was performed by CLIA method using the Abbott Architect (Abbott Park, IL, USA). Only results obtained at diagnosis and before therapy initiation were considered for the study. Patients without EBV serology, incomplete charts from patients followed in another center, or final diagnosis other than IBD were excluded from the study.

A retrospective chart review was performed using electronic medical records and the local IBD database in our center. Demographic information and data on disease characteristics at diagnosis were collected, including disease location, behavior, age at onset, presence of growth delay, as well as biochemical markers of disease activity, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal calprotectin, hemoglobin, albumin, and liver function tests where available. Other data collected included treatment received in the first 6 months after diagnosis, clinical symptoms at diagnosis and on follow-up visits, and clinical severity scores as recorded by the treating physician (pediatric Crohn's disease activity index [PCDAI] or pediatric ulcerative colitis activity index [PUCAI], as appropriate).

EBV viral load at the time of IBD diagnosis was retrospectively analyzed using in-house quantitative polymerase chain reaction (qPCR) assay on preserved serum from the time of diagnosis. Sera were stored frozen at −20°C in the serology laboratory for up to 2 years. The serum of the patients who were seropositive from disease onset was retrieved when available and quantitative PCR was performed to assess the viral load of EBV at diagnosis.

Statistical analysis. We used SPSS software to perform descriptive analysis on variables such as demographics, baseline characteristics of disease at onset, age of onset, and treatments used in the seropositive and seronegative groups. We report means and SD, or medians and interquartile range (IQR) for variables with skewed data. We then stratified rates of seropositivity by age group, defining three discrete age categories in keeping with the Paris classification as follows: <10 years, 10 to <17 years, and ≥17 years.[21] We then assessed rates of positive PCR at the time of diagnosis and on follow-up for the overall population. Chi-squared tests were performed for analysis of disease characteristics and age groups in relation to serologic status.

Results

We identified a total of 216 patients aged 0–18 years who were diagnosed with IBD during our 2-year recruitment period. Eighty-six patients had no EBV serology done at the time of diagnosis, four patients had a final diagnosis other than IBD, and six had incomplete charts and were thus excluded from the study, leaving a total of 120 patients in our study population. Among them, 53 (44.2%) were seropositive for EBV IgG (VCA and/or EBNA) and 67 were seronegative (55.8%). Of note, two of these patients were both IgM and IgG seropositive, suggesting possibly active or recent infection at the time of diagnosis.

When comparing disease characteristics between groups, there was a similar incidence of IBD classification: in seronegative patients, 61.2% had CD, 38.8% had UC and IBD-U, and in

seropositive, 69.8% had CD and 30.2% had UC and IBD-U ($P = 0.11$). No association was found between seropositivity and disease severity. In patients with CD, mean PCDAI score was 36 in seronegative patients, compared with 34 in seropositive patients ($P = 0.5$), while patients with UC had a mean PUCAI score of 47 in the seronegative group *versus* 51 in the seronegative ($P = 0.53$). In addition, both groups had similar distribution of biochemical markers of disease severity at diagnosis (hemoglobin, CRP, ESR, and ALT) and disease activity scores. Baseline characteristics of patients in seronegative and seropositive groups can be found in Table 1. When stratified by age group, the prevalence of seropositive patients was for 0 to <10 years 36% ($n = 9$), 10 to <17 years 46% ($n = 40$), and ≥17 years 50% ($n = 4$). Mean age at diagnosis for seropositive patients was 12.4 years for seronegative patients and 12.9 years for seropositive ($P = 0.43$). Only 16 patients had lymphocyte phenotyping as part of their baseline workup.In our center this is done routinely for patients 6 years old and under at the time of IBD diagnosis. In the seronegative patients, 44.5% were normal *versus* 55.6% abnormal, and in the seropositive group, 14.3 *versus* respectively. Lymphocyte phenotyping abnormalities were all mild and nonspecific, and not indicative of specific immunodeficiency.

Overall, therapies started within 6 months of diagnosis were also similar in both the seropositive and seronegative groups, the majority receiving some form of immunosuppression. Within the seropositive group, 66% received systemic corticosteroids, 32.1% infliximab, 5.7% Adalimumab, 5.7% azathioprine, 15.1% received methotrexate, 28.3% aminosalicylates, and 32.1% exclusive enteral nutrition, *versus* 61.2, 47, 6.2, 10.4, 15.2, 25.8, and 28.4%, respectively, in the seronegative group. Only 1.5% of all patients received 6-mercaptopurine, and all were seronegative at the time of treatment initiation, reflecting somewhat of an influence of the serology on the choice of thiopurine use. The choice of therapy in seropositive *versus* seronegative IBD patients within the first 6 months is illustrated in Figure 1.

At the time of retrieval, 47 of 53 seropositive patients had available serum from baseline serologic testing done at diagnosis. Viral load as measured by quantitative PCR on preserved serum from the time of diagnosis was undetectable in all seropositive patients tested.

Discussion

The seroprevalence of EBV in a healthy population of 8417 American children has been demonstrated through the National Health and Nutrition Examination Survey, with an overall rate of seropositivity in 66.5% of children aged 6–19 years. Seroprevalence was shown to increase with age, with a rate of seropositivity of 54.1% in children aged 6–8 years and up to 82.9% in 18–19-year-old children.[22] Data from our study demonstrate that

Table 1 Disease and baseline characteristics at diagnosis

	Seronegative (*n*)	Seropositive (*n*)	Total	*P*-value
Total	67	53	120	
Age (Paris classification)				
0 to <10 years	64% (16)	36% (9)	25	
10 to <17 years	54% (47)	46% (40)	87	
≥17 years	50% (4)	50% (4)	8	
Age (mean, years)	12.4	12.9		0.430
Diagnosis				0.110
CD	61.2% (41)	69.8% (37)		
UC	28.4% (19)	13.2% (7)		
IBD-U	10.4% (7)	17% (9)		
Disease severity (CD)				
PCDAI (mean)	36	34		0.498
PCDAI 10–30	37.8% (14)	42.4% (14)	28	
PCDAI 30+	62.2% (23)	57.6% (19)	42	
Disease severity (UC)				
PUCAI (mean)	47	51		0.525
PUCAI 10–30	23.8% (5)	21.4%% (3)	8	
PUCAI 35–60	47.6% (10)	50% (7)	17	
PUCAI 65+	28.6% (6)	28.6% (4)	10	
Biochemical markers (means)				
ALT (U/L)	15.8	16.7		0.891
Hgb (g/L)	111.8	113.6		0.597
Albumin (g/L)	32.14	32.44		0.799
CRP (mg/L)	24.2	22.7		0.805
Lymphocyte phenotype				
Normal	44.5% (4)	14.3% (1)	5	
Abnormal	55.6% (5)	85.7% (6)	11	

ALT, alanine aminotransferase; CD, Crohn's disease; CRP, C-reactive protein; Hgb, hemoglobin; IBD-U, inflammatory bowel disease-unclassified; PCDAI, pediatric Crohn's disease activity index; PUCAI, pediatric ulcerative colitis activity index; UC, ulcerative colitis.

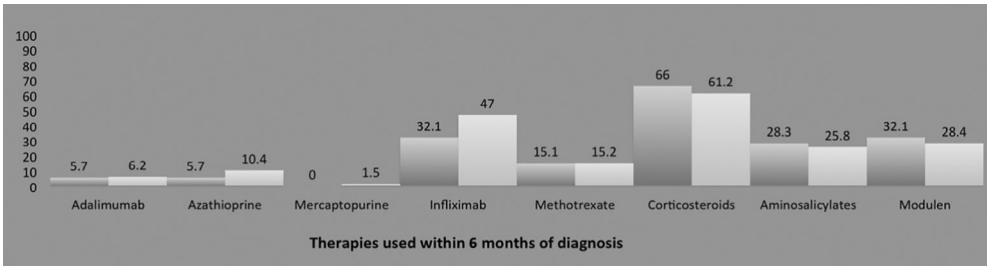

Figure 1 Therapies used within 6 months of diagnosis. (□), Seronegative; (■), seropositive.

EBV seropositivity is slightly lower in our local pediatric IBD population than that shown in the group of healthy children noted above. As expected, however, our rates of seroprevalence increased with age. In addition, our local seroprevalence of EBV was slightly lower when compared with rates previously reported in children with IBD.[15–17,23] Bachman *et al*. reported a seroprevalence of EBV of 53% in their cohort of 194 children with IBD on serology at the time of diagnosis.[16] Of the seronegative patients, 17% showed a seroconversion at a mean of 4.3 years after diagnosis. They report three patients who developed malignancy, though noted no association with EBV infection.[16] Harris *et al*. reported a seroprevalence at diagnosis of 53%, with 87% of these being treated with thiopurines, as well as 83% of the seronegative patients. None of their patients developed EBV-related complications or LDs over an 8-year period.[17] A multicenter cross-sectional described EBV seroprevalence in 495 patients with IBD, and demonstrated an overall seroprevalence of 72.8%, with, interestingly, 0% in patients under 5 years and less than 60% in patients under 30 years.[24] To our knowledge, these are currently the only studies describing the seroprevalence at the time of diagnosis of IBD in a pediatric population.

We did not demonstrate any impact of serologic status on disease characteristics, such as disease activity at onset as defined by PUCAI or PCDAI scores, or IBD subtype (CD *vs* UC *vs* IBD-U). This suggests that EBV exposure prior to disease onset does not significantly impact the mode of presentation of IBD. Additionally, EBV serologic status did not seem to impact clinical decisions regarding therapy in our center, as suggested by the absence of association of treatments with EBV seropositivity.

In our patient population, at onset of IBD, no patients had positive EBV PCR on serum retrospectively tested for the purpose of this study. Interestingly, six patients in our cohort had EBV viral loads performed on whole blood (WB) during clinical follow-up due to the presence of clinical symptoms consistent with a viral infection. These were performed outside the context of this study and WB was used as a standard of care. A summary of the serologies and qPCRs done in real time on WB on these patients can be found in Table 2. One patient had a positive quantitative PCR at the time of diagnosis, showing evidence of acute infection. Two other patients became positive at 7 and 17 months after diagnosis, indicating viral reactivation. One of these continues to have evidence of chronic active EBV infection on follow-up PCR testing. Both patients had received systemic corticosteroids. Three other patients had EBV viral load measured in the months following diagnosis, which remained negative. We explain the discordance in EBV qPCR results between the WB testing done at the time of diagnosis and the retrospectively tested serum by a lower sensitivity of qPCR on serum when the virus is not in its lytic or active phase. In vitro studies show that viral DNA remains detectable for a shorter time in serum than in WB during acute infection,[25,26] suggesting these patients might have been nearing the end of their active infection. Also, DNA conserved at −20°C may have degraded over time, highlighting the fact that low viral loads may have been undetected.

No longitudinal studies, to our knowledge, are currently published reporting the rates of reactivation or persistence of EBV in seropositive children with IBD and their relationship with immunosuppressive therapies. As noted, Hradsky *et al*. demonstrated a positive association between viral load and anti-TNF therapy as well as increased risk for early seropositivity in patients treated with azathioprine.[15] In a pilot study comparing EBV-lytic activity in mononuclear cells of patients with IBD on anti-TNF with healthy controls and patients with IBD not on immunosuppressive therapies, Lapsia *et al*. demonstrated a higher EBV viral

Table 2 Summary of patients with Epstein–Barr virus (EBV) measured on follow-up

Patient	EBV VCA IgG	EBV VCA IgM	EBV EBNA	Viral load at diagnosis (WB)	Viral load at follow-up (WB)	Therapy in the first 6 months
1	Positive	Negative	—	0	3.27 log	Corticosteroid, anti-TNF
2	Positive	Positive	Positive	0	2.65 log	Corticosteroid, EEN
3	Positive	Negative	Positive	0	0	Corticosteroid, 5-ASA
4	Positive	—	—	0	0	Anti-TNF
5	Positive	—	—	0	0	Corticosteroid, EEN
6	Positive	Positive	Negative	4.35 log	0	5-ASA

5-ASA, 5-aminosalicylic acid; anti-TNF, antitumor necrosis factor; EBNA, Epstein–Barr nuclear antigen; EBV, Epstein–Barr virus; EEN, exclusive enteral nutrition; VCA, viral capsid antigen; WB, whole blood.

load and increased transcripts of EBV-lytic genes in patients on anti-TNF agents.[27] Clinically, this may translate to an increased risk of reactivation or persistence of EBV in these patients, thus putting them at risk for lymphoproliferative complications. In adult patients with IBD, higher rates of seropositivity and more elevated EBV viral loads have been reported in patients treated with anti-TNF agents.[28]

Concerns remain about the potential for EBV complications in both seronegative patients who may develop primary EBV infection on IBD therapies as well as in previously exposed, seropositive individuals, including the risk of clinically apparent viral reactivation, persistence of elevated viral loads or chronic active EBV, and relationship to disease flare-ups as well as lymphoproliferative complications. Further prospective cohort studies are needed to follow EBV viral load from disease onset in pediatric patients with IBD.

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